Soil Microbial Responses to Different Precipitation Regimes Across a Southwestern United States Elevation Gradient

by

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ABSTRACT

Soil organic carbon (SOC) is a critical component of the global carbon (C) cycle, accounting for more C than the biotic and atmospheric pools combined. Microbes play an important role in soil C cycling, with abiotic conditions such as soil moisture and temperature governing microbial activity and subsequent soil C processes. Predictions for future climate include warmer temperatures and altered precipitation regimes, suggesting impacts on future soil C cycling. However, it is uncertain how soil microbial communities and subsequent soil organic carbon pools will respond to these changes, particularly in dryland ecosystems. A knowledge gap exists in soil microbial community responses to short- versus long-term precipitation alteration in dryland systems. Assessing soil C cycle processes and microbial community responses under current and altered precipitation patterns will aid in understanding how C pools and cycling might be altered by climate change. This study investigates how soil microbial communities are influenced by established climate regimes and extreme changes in short-term precipitation patterns across a 1000 m elevation gradient in northern Arizona, where precipitation increases with elevation. Precipitation was manipulated (50% addition and 50% exclusion of ambient rainfall) for two summer rainy seasons at five sites across the elevation gradient. *In situ* and *ex situ* soil CO_2 flux, microbial biomass C, extracellular enzyme activity, and SOC were measured in precipitation treatments in all sites. Soil CO_2 flux, microbial biomass C, extracellular enzyme activity, and SOC were highest at the three highest elevation sites compared to the two lowest elevation sites. Within sites, precipitation treatments did not change microbial biomass C, extracellular enzyme activity, and SOC. Soil CO_2 flux was greater under precipitation addition treatments than exclusion treatments at both the highest elevation site and second lowest elevation site. *Ex situ* respiration differed among the precipitation treatments only at

the lowest elevation site, where respiration was enhanced in the precipitation addition plots. These results suggest soil C cycling will respond to long-term changes in precipitation, but pools and fluxes of carbon will likely show site-specific sensitivities to short-term precipitation patterns that are also expected with climate change.

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INTRODUCTION

Drylands, defined by a ratio of mean annual precipitation to potential evapotranspiration of less than 0.65, cover 45% of the earth's terrestrial surface and are projected to cover 50 to 56% of land area by the end of the twenty-first century (Huang et al., 2016; Prăvălie, 2016). The expansion of drylands is mainly due to increased temperature and reduced precipitation (Prăvălie, 2016). Dryland soils have a low soil organic carbon content, comprising around 1% of soil mass, as opposed to mesic forest soils, where organic carbon comprises about 5% of soil mass (Huang et al., 2016). Despite low dryland soil organic carbon, these systems contain around 15% of the global soil organic carbon due to the large area they cover (de Graaff et al., 2014; Lal, 2004; Talmon et al., 2011). The soil carbon pool in drylands is constrained by soil moisture, with greater soil carbon storage typically corresponding with more precipitation (Conant et al., 1998; Talmon et al., 2011).

Within the soil organic carbon pool, soil microbes play an important role in carbon cycling, and these microbial communities are strongly affected by soil moisture, particularly in dry systems. In a global analysis of soil microbial biomass, Xu et al. (2013) found that drylands are the biome with the lowest microbial biomass, due to low soil moisture and low soil organic carbon. Bacteria have a stronger response to changes in precipitation than fungi, due to their reliance on water for transport (Bell et al., 2009; Schimel et al., 2007). Bacteria are seasonally more abundant in drylands during seasons with increased soil moisture (Bell et al., 2009; Manzoni et al., 2012). Fungal biomass does not respond as quickly as bacteria to changes in soil moisture (Bell et al., 2009; Manzoni et al., 2012; Schimel et al., 2007). This results in overall increases in bacterial biomass both during years with more precipitation as well as under extreme short-term, precipitation addition treatments (Bell et al., 2009).

Fungi have the ability to produce extracellular enzymes to facilitate decomposition. Only certain fungi can produce certain extracellular enzymes, and microbial communities rely on these enzymes to breakdown complex organic compounds (Feng et al., 2019; Ladwig et al., 2015; Sinsabaugh et al., 2008). Fungal production of extracellular enzymes is dependent on soil moisture as well as the quality and quantity of soil organic matter present (Bell et al., 2009). Additionally, extracellular enzyme activity is not linearly correlated with soil moisture. At mesic sites, enzyme activity is stable with increasing aridity, but enzyme activity rises exponentially with increasing aridity at drier sites (Feng et al., 2019). Since vegetated areas have greater soil moisture and soil organic matter than interspace areas, microbial biomass is typically enhanced in vegetative patches relative to nonvegetative patches (Ladwig et al., 2015; Talmon et al., 2011).

Soil respiration, a combination of respiration due to roots and microorganisms living in the soil, is one of the main pathways where carbon is exchanged between the atmosphere and terrestrial ecosystems (Liu et al., 2009).Soil respiration is constrained by soil moisture and follows trends similar to those seen with carbon storage. Two reviews of global soil respiration found the lowest rates of respiration in dryland ecosystems and highest respiration rates in tropical forests, where temperature and soil moisture are high year-round (Raich and Schlesinger, 1992; Schlesinger and Andrews, 2000). Higher soil respiration rates typically correspond with ecosystems that have greater mean annual precipitation, due to soil moisture limiting decomposition rates in arid soils (Chatterjee and Jenerette, 2011; Conant et al., 2004; Liu et al., 2009; Wilson and Griffin, 1975). Under short-term precipitation manipulation, soil respiration increases in drylands with precipitation addition as a response of stimulated microbial biomass (Liu et al., 2009; Shen et al., 2009; Talmon et al., 2011).

Climate models predict that there will be less rainfall and warmer temperatures in the next century in the American Southwest. Extreme short-term reductions in precipitation will lead to lower levels of soil moisture, leaving regions like the American Southwest more arid (Seager et al. 2007). Future increases in drylands extent, reductions in precipitation, and the sensitivity of soil microorganisms to changes in soil moisture lead to the question: How do short- and long-term changes in precipitation alter soil microbial community processes in dryland systems?

Both long-term (historical), established precipitation patterns and short-term (recent years) climate change influence plant communities, biomass, and traits as a result of changes in precipitation. However, precipitation impacts other aspects of ecosystems, such as soil organic carbon and microbial communities. The spatial location of sampling relative to plants also affects carbon storage in the soil, and soil carbon pools have been shown to be greater in vegetated areas than in inter-canopy areas, where there is no immediate input due to plants (Conant et al., 1998). While annual respiration rates indicate a stronger dependence on precipitation than soil temperature, monthly soil respiration rates are more dependent on soil temperature (Conant et al., 1998).

To understand how soil microbial communities will respond to precipitation variation, I used an elevation gradient as a proxy for long-term changes in climate and measured soil organic carbon, soil and microbial respiration, microbial biomass, and extracellular enzyme activity. Due to the strong connections between climate, soil organic carbon, and microbial communities, I have a systems diagram to show how changes in one variable could cause responses in other variables (Figure 1). The plus sign represents the positive correlation between two variables, connected by an arrow. For example, higher elevation sites receive more precipitation and have

greater richness and density of the plant communities, resulting in a more diverse and plentiful carbon source, increasing microbial biomass. I used manipulated precipitation treatments to represent short-term climate change with ambient conditions along the gradient to represent long-term climate patterns, as well as canopy and inter-canopy microsites at 0-5 cm and 5-10 cm soil depths, to answer these questions. Understanding how microbial communities respond to climate change, especially in drylands that make up much of Earth's land area and are expected to expand, is essential to understand this relationship between soil microbial communities and plants in response to precipitation variation.

I tested the following hypothesis:

1) There will be greater soil organic carbon at higher elevation sites undergoing precipitation manipulation treatments than at lower elevation sites undergoing precipitation manipulation treatments, due to the greater mean annual precipitation and thus greater plant biomass at higher elevation sites.
2) There will be a smaller response by microbial communities (soil and microbial respiration, microbial biomass, and extracellular enzyme activity) at higher elevation sites undergoing precipitation manipulation treatments than at lower elevation sites undergoing precipitation treatments (Figure 2).
    a) Microbial enzyme activity, microbial biomass, and soil respiration will be more responsive to precipitation manipulation at lower elevation sites than at higher elevation sites due to being more water limited at lower elevation sites.

Study Overview

The US Geological Survey's Gradient Rainfall Manipulation Project (GRaMPS) manipulates rainfall across an elevation gradient in northern Arizona to explore the impact of extreme precipitation change with long-term precipitation trends. GRaMPS uses rainout shelters to intercept rainfall on one-third of the plots, uses sprinklers to redistribute the collected water on one-third of the plots, and leaves rainfall in one-third of the plots unmanipulated as controls. The manipulated precipitation represents immediate, short-term extreme changes in precipitation while the naturally occurring precipitation differences across the elevation gradient represent changes in long-term precipitation trends. I used the established GRaMPS sites and precipitation treatments to assess how soil microbial communities in northern Arizona change with long-term precipitation patterns and with short-term precipitation manipulation. This project offers a unique opportunity not only to assess how microbial communities respond to manipulated precipitation, but also to understand how current microbial communities differ from one another along an elevation gradient.

METHODS

Site Description

The five GRaMPS sites increase in mean annual precipitation, clay and silt content, and vegetative cover, and decrease in mean annual temperature, as elevation increases (Figure 3, Table 1). The vegetation at the three highest sites includes conifer trees, however the GRaMPS plots are in open, grassy meadows. The two lowest sites, Black Point and Antelope, both have *Bouteloua eriopoda* (black grama) as the dominant grass species and the two highest sites, Arboretum and Camp Colton, both have *Festuca arizonica* (Arizona fescue) as the dominant grass species. The mid-elevation site, Blue Chute, is the only site where the dominant grass species is *Bouteloua gracilis* (blue grama).

Every site contains twelve 2 m X 3 m plots with each plot randomly assigned to one of three precipitation treatments, with treatments replicated four times within the site. The treatments are: ambient (no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion), and 50% water addition (addition). The rainout shelters, similar to Yahdjian and Sala (2002), have metal frames holding up clear acrylic bands at a 20° angle with a gutter at the lower end channeling intercepted water into a storage drum. The acrylic bands are deployed in early May before the first monsoon rain and removed in early October before the first snowfall at the highest sites. The acrylic bands are spaced to intercept 50% of precipitation (Figure 4). Sprinklers redistribute the collected water on rainfall addition plots (Figure 5). The rainout shelters were installed prior to the summer 2016 monsoon rains; this study took place after two growing seasons of treatment.

I measured soil respiration during the months of June, July, August, and September 2018. There were three sampling campaigns during the pre-monsoon period (4-6 June, 18-21 June, and 2-5 July), two sampling campaigns during the

peak-monsoon period (23-26 July and 14-17 August), and one sampling campaign during the post-monsoon period (11-14 September). For assessing microbial biomass, microbial extracellular enzymes, and soil organic carbon I took soil samples from July 30th to August 1st, 2018.

Soil Sampling

I collected soil cores using a steel tube (7 cm diameter) at depths of 0-5 cm and 5-10 cm adjacent to the canopy of the dominant grass species and in the inter-canopy between the dominant grass species. Canopy was defined as locations under the canopy of grass while inter-canopy was defined as open spaces between dominant grass. Spacing of intercanopy sites were different for each site, with larger intercanopy spaces at lower elevation sites than at higher elevation sites due to greater plant density at higher elevation sites. Canopy and inter-canopy are referred to as "microsites". I homogenized 2 cores per plot depth and microsite, resulting in 240 soil samples (N = 1 homogenized sample per plot depth and microsite x 2 depths per plot x 2 microsites per plot x 12 plots per site X 5 sites = 240 soil samples). This was done at peak monsoon season, during the growing season, to capture microbial communities at peak activity. I sampled at two depths to access microbial communities in the surface soils and microbial communities that live farther down in the soil, where there is a greater buffer against changes in soil temperature and soil moisture than at the surface, as well as less carbon inputs. Soil samples were passed through a 2 mm sieve and the <2 mm soil fraction was air dried before being allocated for microbial biomass, microbial extracellular enzymes, and microbial respiration analyses.

Soil Organic Carbon Measurements

I analyzed soil samples for organic carbon concentration. I ground the soil using a ball mill (8000D, Spex CertiPrep, Metuchen, NJ, USA) and acid fumigated soil subsamples to remove inorganic carbon in the form of carbonates that might be in the soil (Harris et al., 2001). The carbon content of the subsamples was measured in duplicate replications using an elemental analyzer (ECS 4010, Costech Analytical, Valencia, CA, USA).

In Situ and Ex Situ Soil Respiration

To understand the stability of soil carbon in the system, I coupled *in situ* and *ex situ* soil respiration measurements. In the field, I used a portable gas exchange system with a soil chamber attachment (Li-Cor 6400-09, Licor, Lincoln, NE, USA) to measure soil respiration in adjacent canopy and inter-canopy (see soil sampling methods for microsite descriptions) locations.

A week prior to the first respiration measurements, I installed two PVC collars (10 cm diameter and 4.4 cm height) per plot for canopy and inter-canopy into the soil at about 2.2 cm depth where I would be sampling soil respiration. I measured soil respiration, temperature, and relative humidity with a portable gas exchange system. Per sampling campaign, I took four replicate respiration measurements on each collar, for a total of approximately 2,160 respiration replicates. Each collar had its replicate measurements averaged per sampling campaign, resulting in 24 respiration rates per collar across all 6 sampling times.

To account for root respiration, I used laboratory microcosms to determine a more controlled respiration rate of soil without roots to compare to the field results. Each microcosm contained approximately 50 g of air-dried soil placed on top of an ashed glass microfiber filter (GF/F) on top of 40 g of 1 mm diameter glass beads in a

glass mason jar (473 ml). To maximize microbial activity, I wetted soil to 60% water holding capacity. After sealing the jars, I extracted 10 mL of headspace CO_2 using a syringe and promptly injected the gas sample into a CO_2 gas analyzer (Li-Cor 7000, Licor, Lincoln, NE, USA). I used certified CO_2 standards (4980 ppm) to create a calibration curve to calculate CO_2 from the injected value. When headspace CO_2 concentration levels were higher than 5000 ppm, I flushed jars with ambient air by opening each jar and fanning them for four minutes. After a jar had five flushing events, I rewetted the jar to maintain 60% water holding capacity (Robertson et al., 1999). Using the Ideal Gas Law, I converted CO_2 concentrations (ppm) to mass (μg CO_2-Carbon) and then normalized the converted values by organic carbon present in the soil (Robertson et al., 1999). I expressed CO_2 respiration rates as the change in CO_2 concentration per unit soil dry mass divided by the time elapsed between sampling points.

Microbial Biomass

Soil microbial community size is difficult to determine by direct observation through microscopes when communities are large, so instead, microbial biomass can be extracted from soil in the form of dissolved organic carbon. This is the optimal method for large samples (Paul et al., 1999). I did this for all of my samples following the methods of Jenkinson (1966) with modifications by Gravuer and Eskelinen (2017). Soils were fumigated with chloroform for 48 hours to lyse microbial cells. Each sample had a fumigated extraction, to determine microbial dissolved organic carbon, and unfumigated extraction, to determine baseline dissolved organic carbon. I used a rotary shaker to disperse 10 g of soil and 50 mL 0.5 M K_2SO_4 for 1 h before filtering through Whatman #1 filter paper. Dissolved organic carbon was measured with a Shimadzu Total Organic Carbon Analyzer (TOC-

V, Shimadzu Corporation, Kyoto, Japan). Dissolved organic carbon was then converted to microbial biomass by dividing the results by an extraction constant, 0.45, calculated by Jenkinson et al. (2004).

Microbial Extracellular Enzymes

I assessed extracellular enzyme activity in order to understand how extracellular enzyme secretions might change across the sites and treatments by measuring the activities of β-1,4-glucosidase, phosphatase, β-1,4-N-acetylglucosaminidase, and cellobiohydrolase. These enzymes were selected for the function they serve to the fungi that produce them with β-1,4-glucosidase releasing glucose from cellulose, phosphatase releasing inorganic phosphate from organic matter, 4-N-acetylglucosaminidase degrading chitin, and cellobiohydrolase releasing disaccharides from cellulose (Allison and Jastrow, 2006). I followed the methods of Sinsabaugh et al. (1999) with modifications, using 4-MUB-β-D-glucopyranoside as a substrate for β-1,4-glucosidase, 4-MUB-phosphate for phosphatase, 4-MUB-N-acetyl-β-D-glucosaminide for β-1,4-N-acetylglucosaminidase, 4-MUB-B-D-cellobioside for cellobiohydrolase. I tested enzyme activities for all treatment types for the top 0-5 cm depth of canopy soil only. Concentrations and incubation periods were determined through a trial for maximum activity (Personal Communication: Leslie Nichols, January 8, 2019) and differed among substrates. To determine substrate concentrations and incubation times, I selected three control samples and added four concentrations of each substrate and incubated for 1, 2, 4, and 6 hours. I determined the ideal concentration and incubation time for each substrate after reading the trial plates and determining which combination had the highest activity time. Using the information from this trial, I measured activities of the preciously listed enzymes. Fluorescence of substrate plates were read at an excitation

wavelength of 360 nm and an emission wavelength of 460 nm using a microplate reader (BioTek SynergyH1, Winooski, VT, USA).

Data Analysis

I assessed differences in *in situ* respiration, microbial biomass carbon, enzyme activity, and soil organic carbon using analysis of variance (ANOVA) and assumed significance of $P < 0.05$. For *in situ* respiration, I used a four-way (ANOVA) with site, treatment, sampling season, and microsite as fixed effects. I also used linear models to investigate the effect of soil temperature and volumetric water content on *in situ* respiration. For *ex situ* respiration rates, I used a locally estimated scatterplot smoothing (LOESS) regression with a span of 1 and a two-way ANOVA with site and treatment as fixed effects. I assessed microbial biomass carbon using a four-way ANOVA with site, treatment, microsite, and depth as fixed effects. I assessed the activity of each enzyme using a two-way ANOVA with site and treatment as fixed effects. For enzyme activity, I also compared sites and enzymes using nonmetric multidimensional scaling (NMDS). I assessed soil organic carbon using a four-way ANOVA with site, treatment, microsite, and depth as fixed effects. I used the Tukey-Kramer test for all *post hoc* analyses. I performed all of my data analyses using R (Version 3.3.1, R Core Team, 2016).

RESULTS

Soil Organic Carbon

Soil organic carbon concentration by mass differed with site ($F_{4, 51}$ = 429.9, P < 0.001) and generally increased with increasing elevation (Figure 6). Camp Colton, the highest elevation site with the greatest mean annual precipitation, had greater soil organic carbon than the other sites. There was greater soil organic carbon at the depth of 0-5 cm than at 5-10 cm ($F_{1, 51}$ =12.8, P < 0.001) while there were marginally significant increases in soil organic carbon in the canopy than inter-canopy microsites ($F_{1,51}$ = 3.3, P = 0.076). The precipitation manipulation treatments did not have a significant effect on soil organic carbon ($F_{2,51}$ = 0.3, P = 0.8). There were no significant interactions.

In Situ and Ex Situ Respiration

A four-way ANOVA indicates that site ($F_{4,263}$ = 209.0, P < 0.001) was a significant main effect for *in situ* soil respiration. The two lowest elevation sites, Black Point and Antelope, had lower soil CO_2 efflux than the three higher elevation sites. Precipitation manipulation had a significant effect on soil respiration ($F_{2,263}$ = 13.0, P < 0.001). Precipitation addition plots had higher soil CO_2 efflux than both exclusion and control treatment plots (Figure 7). Control treatment plots did not have higher efflux than exclusion treatment plots. Soil CO_2 efflux measurements taken both during monsoon season and immediately after monsoon season were significantly higher than measurements taken before monsoon season ($F_{2,263}$ = 41.9, P < 0.001), but were not significantly different from one another. Microsite ($F_{1,263}$=0.5, P = 0.49) was not significant (Figure 7) and there were no significant interactions.

Soil CO_2 efflux was positively related to volumetric water content (Figure 8). CO_2 efflux at Camp Colton was higher for all volumetric water content than for the four lower elevation sites, however CO_2 efflux increased with volumetric water content the same across all sites. The linear regression of CO_2 efflux vs volumetric water content for Camp Colton had a slope of 9.2 while the slope for the linear regression of lower four sites was 8.4 (Figure 8).

A two-way ANOVA indicates that site ($F_{4,797}$ = 3.1, P = 0.015) was a significant main effect on *ex situ* respiration rates. Most sites did not differ from one another, but *ex situ* respiration was lower at Blue Chute, the mid elevation site, than at Black Point, the lowest elevation site. Treatment ($F_{2,797}$= 0.6, P = 0.54) was not significant. There were significant interactions between site and treatment ($F_{8,797}$= 2.4, P = 0.017) but only with reduced respiration rates in Black Point precipitation exclusion samples compared to Blue Chute control and precipitation exclusion samples. LOESS regressions indicate *ex situ* respiration rates decreased over time (Figure 9).

Microbial Biomass

Microbial biomass carbon for soils sampled during peak monsoon season at two depths (0-5 cm and 5-10 cm) and at two the microsites (canopy and inter-canopy) generally peaks mid-elevation (Figure 10). Results from a five-way ANOVA show microbial biomass carbon differed among sites ($F_{4,\ 223}$ = 68.0 P < 0.001). Black Point and Antelope, the two lowest elevation sites, had less microbial biomass carbon in the sampled soil than the three higher elevation sites, Blue Chute, the Arboretum, and Camp Colton. Additionally, microbial biomass carbon was greater in soil at 0-5 cm than 5-10 cm depth ($F_{1,\ 223}$ = 23.24 P < 0.001) and was greater in the canopy microsite than inter-canopy microsite ($F_{1,\ 223}$ = 8.0 P = 0.005). Microbial biomass

carbon did not differ among precipitation treatments ($F_{2,\ 223}$ = 2.4 P = 0.09). There were no significant interactions.

Extracellular Enzymes

Enzyme activity for soils sampled during peak monsoon season from the top 5 cm of soil in the grass canopy generally increased with elevation (Figure 11). Precipitation manipulation treatments did not change the activities of any enzyme within sites (Table 2). β-1,4-glucosidase activity differed significantly across sites ($F_{4,8}$ = 90.5, P < 0.001) with lowest activity levels at Black Point and highest activity levels at the Arboretum and Camp Colton (Figure 11A). β-1,4-N-acetylglucosaminidase activity differed significantly across sites ($F_{4,8}$ = 99.1, P < 0.001) with lowest activity levels at Black Point and Blue Chute and highest activity levels at the Arboretum and Camp Colton (Figure 11B). Cellobiohydrolase activity was different across sites ($F_{4,8}$ = 243.0, P < 0.001) with Black Point having the lowest CBH activity (Figure 11C). Finally, phosphatase was also different across sites ($F_{4,8}$ = 29.5, P < 0.001) with lowest activities at Black Point and Antelope and highest activities at the Arboretum and Camp Colton (Figure 11D). The NMDS shows that there is overlap between enzyme activity at the sites (Figure 12). The profile of the enzymes shows there is no distinct pattern among sites, even with different climates and plant communities across sites (Figure 12). While the total activity of each enzyme changes with elevation, the relative amount of activity is approximately the same.

DISCUSSION

Soil Organic Carbon

The positive relationship between soil organic carbon and mean annual precipitation supports my hypothesis that soil organic carbon would increase with elevation. Talmon et al. (2011) conducted a similar study across an aridity gradient in Israel and also found a positive correlation between soil organic carbon and mean annual precipitation. They found higher organic carbon in soil in vegetation patches and differences with depth but did not find differences among 30% precipitation addition and reduction treatments. Their results match mine with soil organic carbon responses to site, microsite, and depth, but no responses to precipitation manipulation. Shi et al. (2014) found that over a 50-year period, there were greater reductions in soil organic carbon due to drought which subsequently reduced soil respiration. My study only had compounding effects of precipitation manipulation for two years so with precipitation manipulation in my study did not significantly alter soil organic carbon, soil organic carbon might shift among precipitation manipulation treatments in the future as the extreme manipulation becomes more long-term. If future monsoon precipitation decreases as predicted by some climate models, plant communities will respond with reductions in above- and belowground biomass and litter inputs (Pascale et al., 2017; Seager et al., 2007, GRaMPS unpublished data).

Currently, GRaMPS aboveground plant biomass research is showing reduced plant biomass in exclusion plots at all sites except the highest elevation site (Munson et al., 2018). One year after precipitation manipulation, the lowest three sites showed less plant biomass in exclusion plots and after three years of excluding summer rain, the four lowest sites are showing less plant biomass in exclusion plots. GRaMPS already has significantly less aboveground biomass under precipitation exclusion plots. These reductions could show an immediate increase in soil organic

carbon through short-term increases in litter inputs through above- and belowground die-off, but if new plant material is not established, then there might be long-term reductions in soil organic carbon.

In Situ and Ex Situ Respiration

In support of my hypothesis, *in situ* respiration increased with increasing elevation. Studies in drylands in Arizona, Israel, and China have all shown soil respiration increases with mean annual precipitation (Conant et al., 1998; Talmon et al., 2011; Zhang et al., 2013). Each of these papers proposed different mechanisms for driving soil respiration with mean annual precipitation. Wetting dry soil exposes soil organic carbon and microaggregates to microbial decomposition as well as promoting increased growth and metabolism of microbial communities, therefore increasing respiration with precipitation (Talmon et al., 2011). Microbial physiology and community structure shift with climate change and regulate soil carbon loss differently, due to the tolerance of water fluctuations by fungal communities (Schimel et al., 2007; Zhang et al., 2013). While I did not perform any molecular analyses on my soil samples and cannot say if the community structures are different across sites, it is possible that dissimilar microbial communities across the elevation gradient result in different soil respiration. Talmon et al. (2011) found lower respiration rates during the dry season, which are comparable to my pre-monsoon measurements and my results follow the same pattern of lower respiration during seasons of less precipitation. Talmon et al. (2011) also measured higher respiration in vegetated patches than in interspaces, which were counter to my results potentially due to Talmon et al. (2011) studying desert shrublands while I studied desert glasslands.

In situ respiration was significantly higher under precipitation addition treatments than ambient and reduced precipitation conditions, however *in situ* respiration for soils undergoing drought treatments conditions did not differ from ambient conditions. Zhang et al. (2013) also found increased rates of respiration in soil undergoing precipitation addition treatments when compared to ambient conditions. This could be due to adaptations by microbial communities to drought conditions, making them resistent to precipitation reductions (Zhang et al., 2013). The lack of response by soil microbial communities in exclusion treatments does not necessarily mean reduced precipitation will have no long-term effect on soil respiration, but the immediate responses might be buffered by other variables such as root biomass and soil organic carbon (Figure 1). For example, roots contribute to soil respiration so if plants continue to allocate energy to producing roots, there will still be soil respiration. It is unknown how long this buffering effect will last, but will be influenced by changes in temperature and precipitation (Weltzin et al., 2003). While site was significant for *in situ* respiration in undergoing precipitation addition treatments, site was not significant for my *ex situ* respiration rate results. This potentially indicates root sources of CO_2 are more sensitive to long-term climate patterns than soil microbial communities.

Microbial Biomass

Microbial biomass increased with elevation, supporting my hypothesis that microbial biomass will increase with mean annual precipitation. This result is similar to findings in other studies. Zhu et al. (2017) found a positive correlation between microbial biomass and long-term soil moisture patterns in arid ecosystems. Maestre et al. (2015) studied 80 global dryland sites, excluding Antarctica, and found reduced microbial abundance in more arid sites. They also found that microbial biomass

increased with soil carbon. I found no microbial biomass responses to the precipitation manipulation treatments, which did not support my hypothesis that microbial biomass would be more responsive to precipitation manipulation at lower elevation sites than at higher elevation sites. My microbial biomass results were also not supported by the literature (Alster et al., 2013; Bell et al., 2009; Lei et al., 2016; Liu et al., 2009). Alster et al. (2013) only reduced precipitation and saw up to 50% reductions in microbial biomass in drought treatments compared to ambient conditions in warm, dry grasslands in Southern California. While this study and my study both reduced precipitation by 50% in the first year, Alster et al. (2013) reduced precipitation by approximately 70% in their study's second year, potentially affecting microbial communities to a greater extent with greater water reduction. Alster et al. (2013) also used a method other than a chloroform fumigation extraction to determine microbial biomass. Liu et al. (2009) found significant increases in microbial biomass in the top 15 cm of soil after three years of precipitation addition in cool, dry grasslands in China. The lengthier study time as well as soil depth could be why my results differed. Bell et al. (2009) found in the Chihuahuan Desert that microbial biomass had a strong positive correlation with short-term changes in monthly soil moisture and microbial biomass was greater during rainy seasons than during dry seasons. I did not measure microbial biomass at different times throughout the year, but I did not see significant changes in microbial biomass due to precipitation manipulation. A synthesis paper on drought in semiarid and arid ecosystems found microbial biomass can be resistant and resilient in a changing environment if there is greater plant diversity and soil organic carbon (Lei et al., 2016). Soil organic carbon did not change with precipitation manipulation treatments, which could contribute to microbial biomass being resistant to the precipitation manipulation treatments.

Microbial biomass was greater in canopy microsites than inter-canopy microsites, as well as greater at a depth of 0-5 cm than 5-10 cm. This supports my hypothesis that microbial biomass would be greater in canopy soil than intercanopy soil as well as greater in soil closer to the surface. Soils surrounding plants generally have higher soil moisture and concentrated pools of labile carbon from decaying plant material and larger microbial communities than in plant interspaces (Austin et al., 2004). This creates more favorable microsites for microbial communities, which are common in drylands where there is large vegetation heterogeneity (Austin et al., 2004; Zhang et al., 2013). Additionally, Zhu et al. (2017) found more microbial biomass in soils sampled from 0-10 cm than in soils sampled from 10-20 cm. This could potentially be due to warmer temperatures and more available organic carbon in soil closer to the surface (Jobbágy et al., 2001). Due to the heterogeneity of drylands, these results could become more noticeable if aboveground plant community biomass changes. Currently GRaMPS aboveground biomass analyses show reductions in biomass under precipitation exclusion plots (GRaMPS unpublished data). Less plant biomass could lead to long-term reductions in soil carbon, limiting resources for microbial communities.

Extracellular Enzymes

Enzyme activities generally increased with elevation, supporting my hypothesis. Feng et al. (2019) found similar patterns of enzyme activity, specifically β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase and phosphatase, declining with increasing aridity across the arid and semiarid grasslands of northern China. Ochoa-Hueso et al. (2018) compared grasslands across North America and Australia and found greater enzyme activity at their mesic sites than their desert sites. They also found that, under short-term drought conditions, enzyme activity increased at mesic

sites, but not at arid sites. Potentially this could occur if there is an accumulation of extracellular enzymes in the soil when soil moisture is low (Ochoa-Hueso et al., 2018). This is not a pattern apparent in my results, as precipitation treatment effects were not significant for any enzyme activity, not supporting the part of my hypothesis that enzyme activity at lower elevation sites would show stronger responses to precipitation manipulation. This could be due to the larger reduction in precipitation in the Ochoa-Hueso et al. (2018) study. In their study, sites had a 66% reduction of precipitation during the growing season while my study had 50% precipitation reduction during the growing season. However, the Ochoa-Hueso et al. (2018) study and my project used soil collected from the top 0-10 cm during the growing season two years after precipitation manipulation treatments began. It could also be because my higher elevation sites had greater soil organic carbon than my lower elevation sites. In the Chihuahuan Desert, Bell et al. (2009) found β-1,4-glucosidase and phosphatase were positively correlated with soil organic matter. I found higher extracellular enzyme activity at sites that had greater soil organic carbon. In a semiarid grassland in southern California, β-1,4-glucosidase, cellobiohydrolase, and β-1,4-N-acetylglucosaminidase activity increased while phosphatase had no change in plots after undergoing two years of drought treatments when compared to ambient rainfall plots (Alster et al., 2013). I found no change in any enzyme activity in response to precipitation manipulation.

My results are consistent with the part of my hypothesis that extracellular enzyme activity will be lower in sites that receive less precipitation annually, but the results do not support my other hypothesis that there will be changes in enzyme activity within sites as a response to short-term precipitation manipulation. The reason extracellular enzymes at my sites did not change with precipitation manipulation could be because soil organic carbon and microbial biomass did not

differ significantly with precipitation manipulation. If microbial communities did not change in size and soil carbon stocks did not differ significantly, microbial communities might not change their production of extracellular enzymes. This suggests long-term enzyme activity is dependent on long-term water availability and soil organic matter, and enzymes potentially persist in pools that are less constrained by short-term climate manipulation.

Overall Responses

While I did not find that short-term precipitation manipulation resulted in more significant changes at lower elevations than higher elevations (Figure 2), my findings did support connections between long-term precipitation and microbial community responses (Figure 1). Site was the most common driver of change across all response variables while the precipitation manipulation had little influence on response variables. This indicates that short-term change in precipitation does not have an immediate effect on the soil microbial community response variables looked at in this study in this system. This does not necessarily mean the soil microbial communities will not change in the future or have not already changed in variables not looked at in this study, such as fungal to bacterial ratios. Turnover time for soil respiration might be shorter than for microbial biomass or soil organic carbon. Precipitation addition treatments at Black Point, the lowest elevation site, and precipitation exclusion treatments at Arboretum, the second highest elevation site, received approximately the same cumulative precipitation from July 2017- July 2018, but the Arboretum exclusion plots consistently had greater respiration rates, enzyme activities, microbial biomass, and soil organic carbon than Black Point addition plots. Differences due to site override responses to short-term precipitation manipulation.

Climate legacies, vegetation communities, and soil texture all could help buffer soil microbial community responses from increases or decreases in annual precipitation.

Future Research

Since this research is part of a large, long-term project, an overarching goal is to combine these belowground results with the aboveground results. Combining below- and aboveground results will provide a more comprehensive understanding of the relationship between soil microbial communities and plant communities in response to precipitation variation. Aboveground treatment effects appear to be traveling up the elevation gradient, and although this is not seen belowground in the variables measured in this study, it would be interesting to understand if the aboveground and belowground patterns relate to one another and if there are other belowground variables, such as genetic diversity, that are shifting.

My study was based on microbial responses after two years of precipitation manipulation; however, GRaMPS will continue with the rainfall addition and exclusion a part of their aboveground research. I propose researchers in the future should look at similar soil variables to see how microbial communities change with accumulating years of precipitation manipulation. The precipitation manipulations showed some marginal effects on soil respiration and some variables could have significant effects after continuous precipitation manipulation. This would allow us to understand the rate that microbial communities are responding to climate change.

Conclusion

I examined how long-term, established precipitation regimes and short-term precipitation manipulation can influence soil microbial communities in dryland ecosystems. The results from this study suggest that soil carbon cycling will respond

to long-term changes in water availability, but pools and fluxes of carbon will likely show site-specific sensitivities to short-term extreme precipitation patterns that are also expected with climate change. Local climate conditions play a large role in regulating soil microbial communities.

Table 1. The latitude, longitude, elevation, vegetation type, dominant grass species, mean annual precipitation (MAP), and mean annual temperature (MAT) for each of the GRaMPS sites across an elevation gradient in northern Arizona.

Site Name	Latitude	Longitude	Elevation (m)	Soil Texture	Vegetation Type	Dominant Grass Species	MAP (mm)	MAT (˚C)
Black Point	35.682	-111.476	1566	Sandy loam	Desert	*Bouteloua eriopoda*	154	13.3
Antelope	35.584	-111.513	1636	Sandy loam	Grassland	*Bouteloua eriopoda*	215	12.5
Blue Chute	35.588	-111.971	1930	Clay loam	Pinyon-juniper	*Bouteloua gracilis*	491	9.6
Arboretum	35.162	-111.731	2179	Clay loam	Ponderosa pine	*Festuca arizonica*	567	7.4
Camp Colton	35.329	-111.730	2591	Loam	Mixed conifer	*Festuca arizonica*	752	6.2

Table 2. Two-way ANOVA table for the activities of the enzymes β-1,4-glucosidase (BG), β-1,4-N- acetylglucosaminidase (NAG), cellobiohydrolase (CBH), and phosphatase (PHOS). The fixed effects were site and treatment. There were no significant interactions between site and treatment.

Enzyme		Degrees of Freedom	Sum of Squares	Mean Squares	F value
BG	Site	4	96494573	24123643	90.4602 ***
	Treatment	2	637096	318548	1.1945 ns
	Residuals	8	2133416	266677	
NAG	Site	4	33162	8290.4	99.1031 ***
	Treatment	2	206	102.8	1.2284 ns
	Residuals	8	669	83.7	
CBH	Site	4	30953355	7738339	242.9996 ***
	Treatment	2	162779	81390	2.5558 ns
	Residuals	8	254761	31845	
PHOS	Site	4	51897236	12974309	29.5344 ***
	Treatment	2	1096306	548153	1.2478 ns
	Residuals	8	3514354	439294	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = not significant

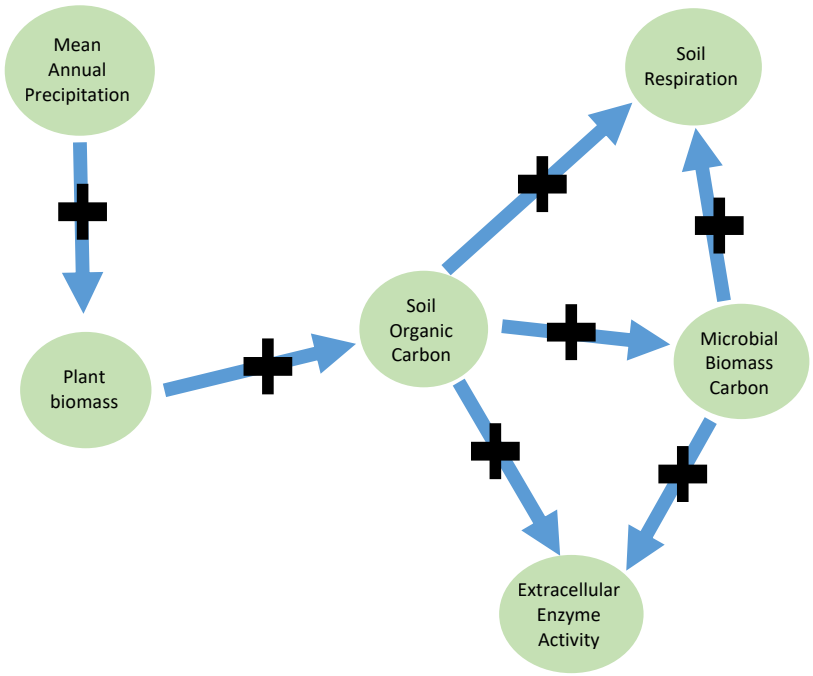

Figure 1. Systems diagram for explanatory and response variables. Variables are mean annual precipitation, plant biomass, soil organic carbon, respiration, microbial biomass carbon, and extracellular enzyme activity. Addition symbols on arrows represent positive correlation between different variables. For example, less mean annual precipitation would lead to less plant biomass.

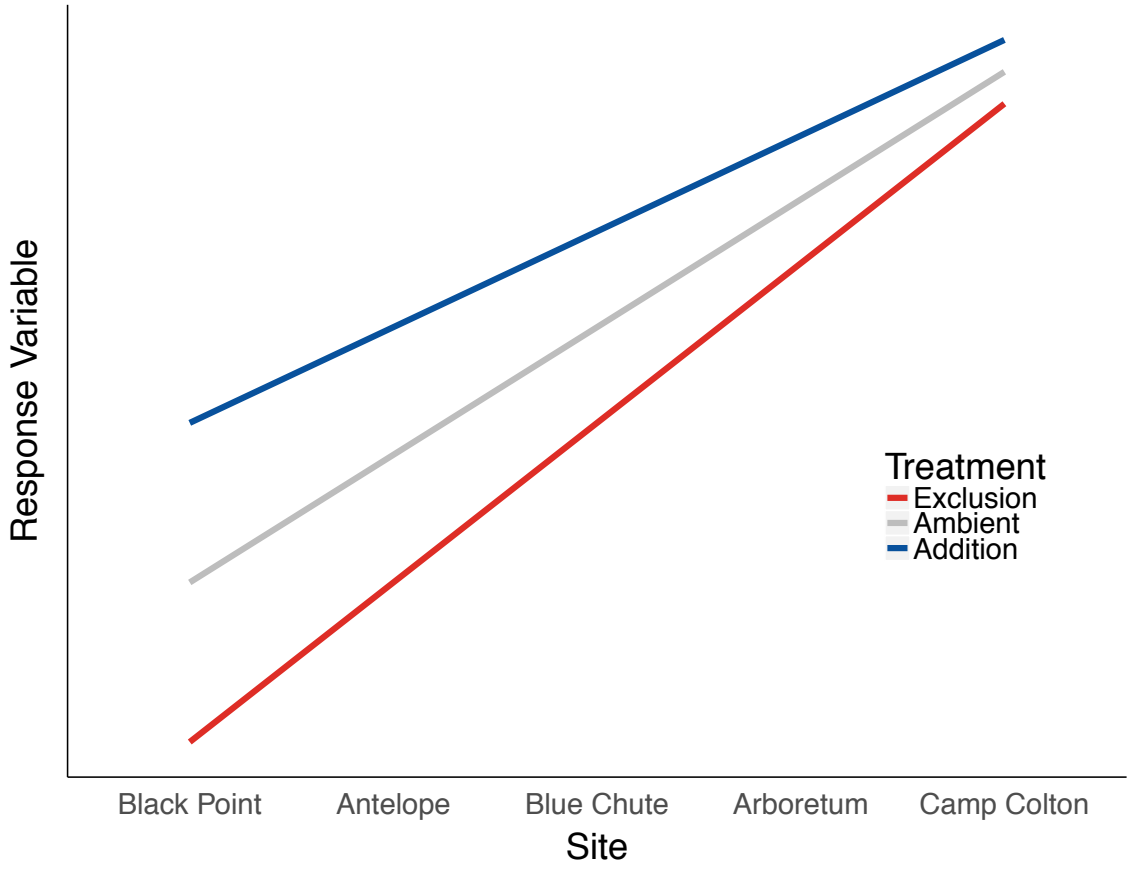

Figure 2. Conceptual graph of hypothesized annual soil respiration responses along the elevation gradient due to precipitation manipulation on plots. Blue is additional precipitation, gray is the control, and red represents exclusion plots.

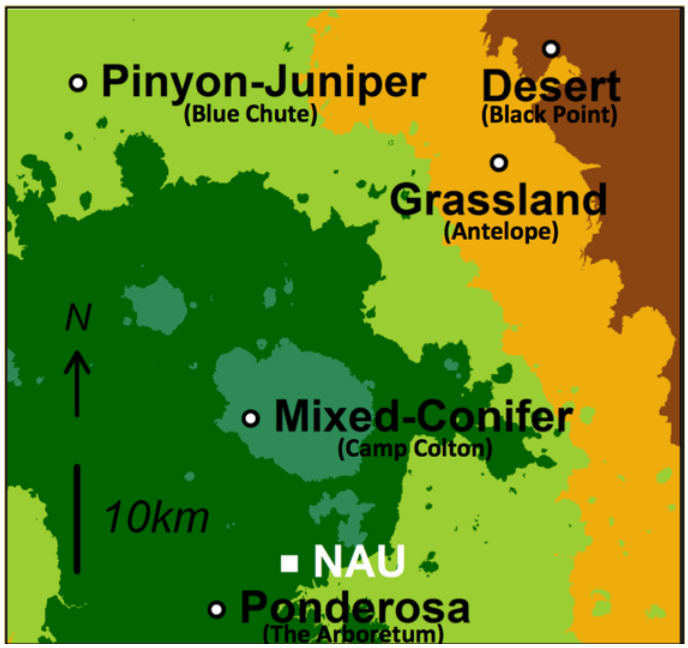

Figure 3. GRaMPS sites and associated plant communities. Color indicates ecosystem range for systems in parentheticals after site name. Sites range from lower elevation drylands to higher elevation forests. NAU is the location of Northern Arizona University. Source: Brad Butterfield

Figure 4. Rainout shelter at the Arboretum and drum for water storage. Rainout shelters intercept approximately 50% of rainfall. Photo by Kaitlyn Toledo.

Figure 5. Sprinklers distributing collected rain water on addition plot at Blue Chute. Photo by Jennifer Gremer.

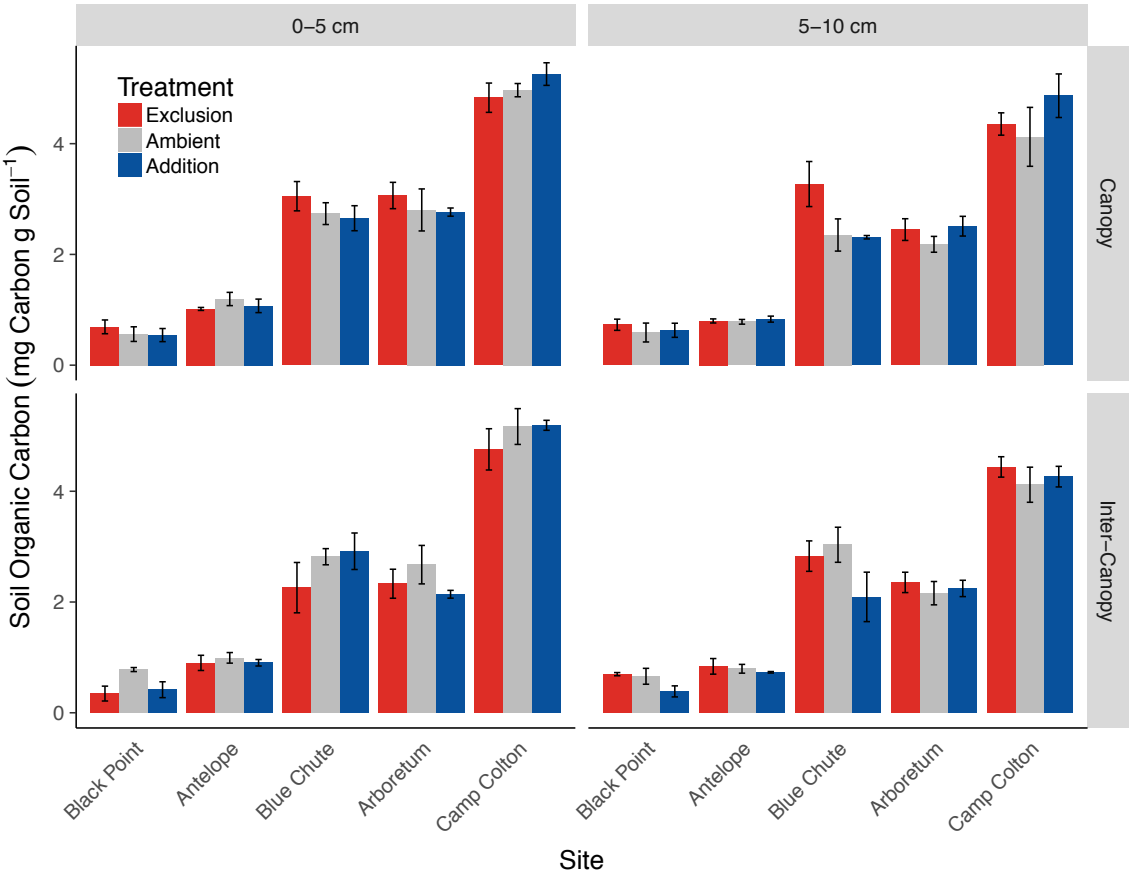

Figure 6. Soil organic carbon (mean ±SE) for each treatment by sites increasing in elevation. Samples were taken in canopy (dominated by grass) and inter-canopy (open spaces between dominant grasses) and at depths of 0-5 cm and 5-10 cm. The precipitation manipulation treatments are: ambient (gray; control; no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion; red), and 50% water addition (addition; blue).

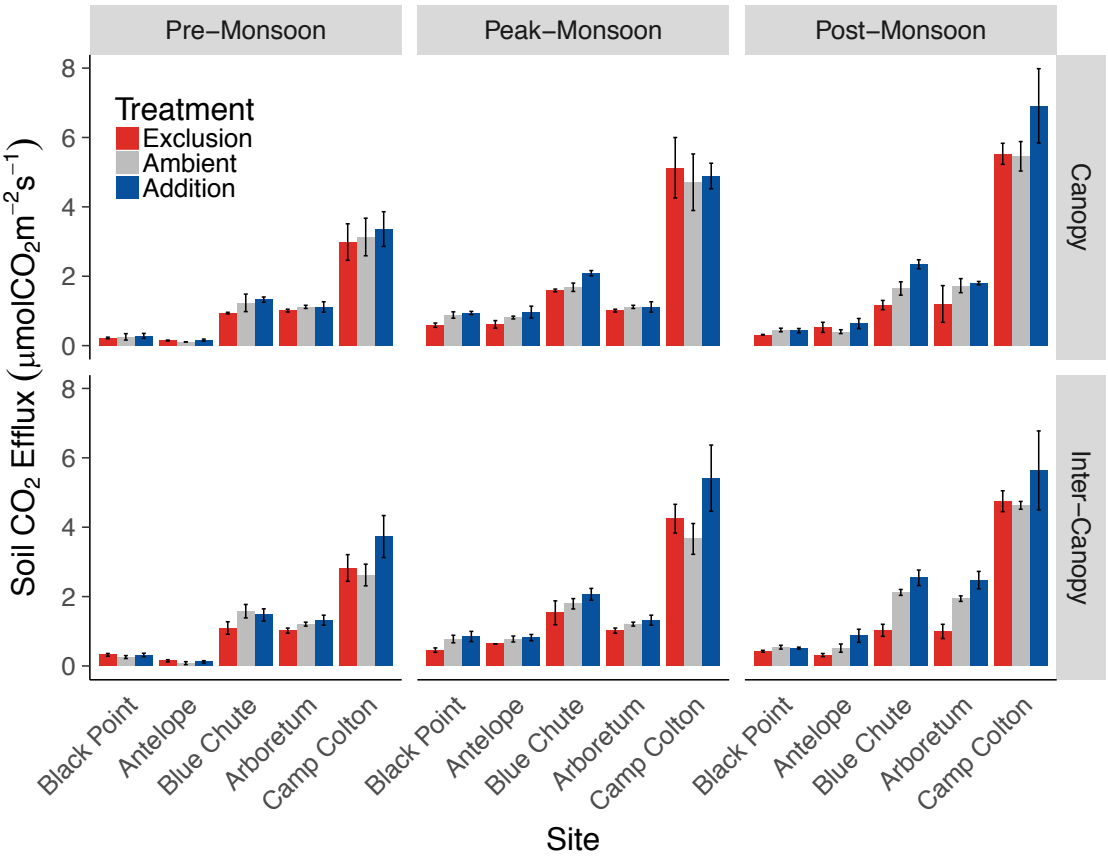

Figure 7. Mean (±SE) Soil CO_2 efflux for each treatment by sites increasing in elevation. Means separated by time in relation to monsoons with "Pre-Monsoon" representing before monsoon season, "Peak-Monsoon" during monsoon season, and "Post-Monsoon" after monsoon season. Measurements were taken at two microsites: canopy, locations dominated by grass, and inter-canopy, open spaces between dominant grass. The precipitation manipulation treatments are: ambient (gray; control; no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion; red), and 50% water addition (addition; blue).

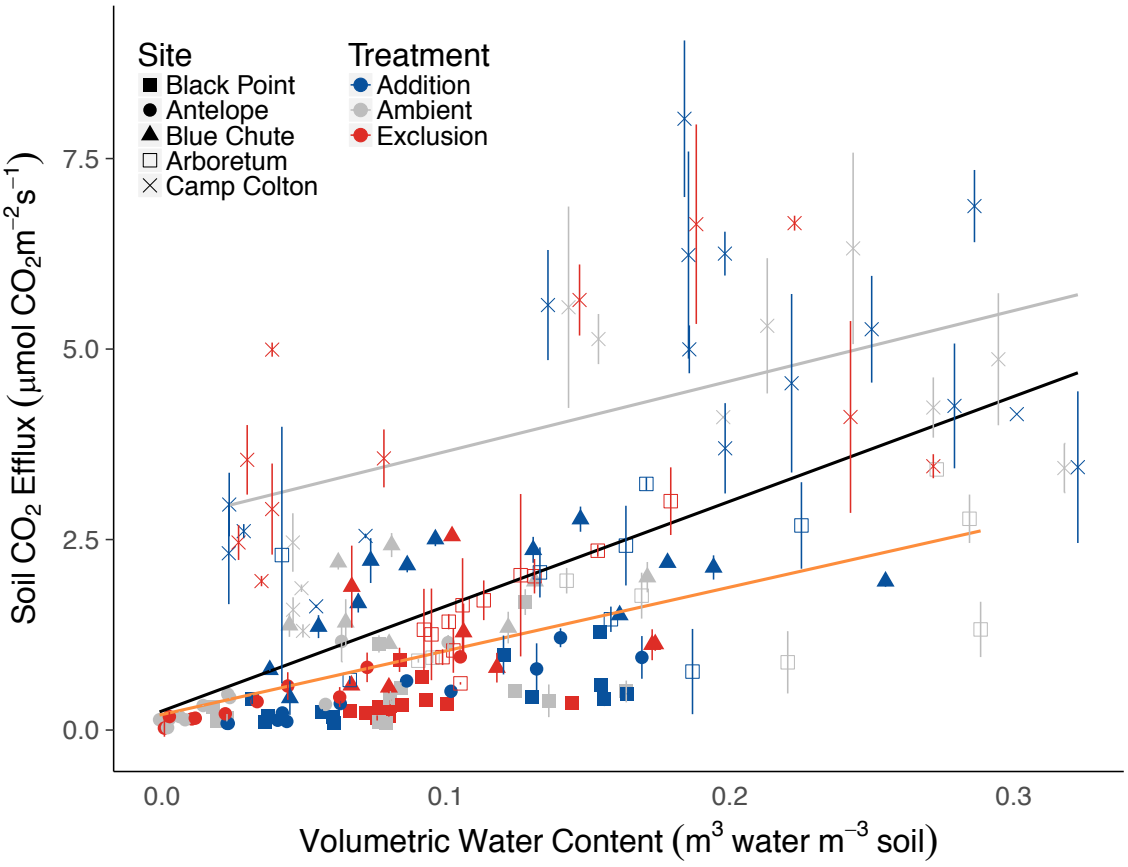

Figure 8. Mean (±SE) Soil CO_2 efflux for each plot across all respiration sampling campaigns at corresponding soil volumetric water content. Microsites were not significant, so measurements were combined. The black linear regression (slope = 13.8) represents all sites, while the gray line (slope = 9.2) represents Camp Colton and the orange line (slope =8.4) represents all sites other than Camp Colton. The precipitation manipulation treatments are: ambient (gray; control; no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion; red), and 50% water addition (addition; blue).

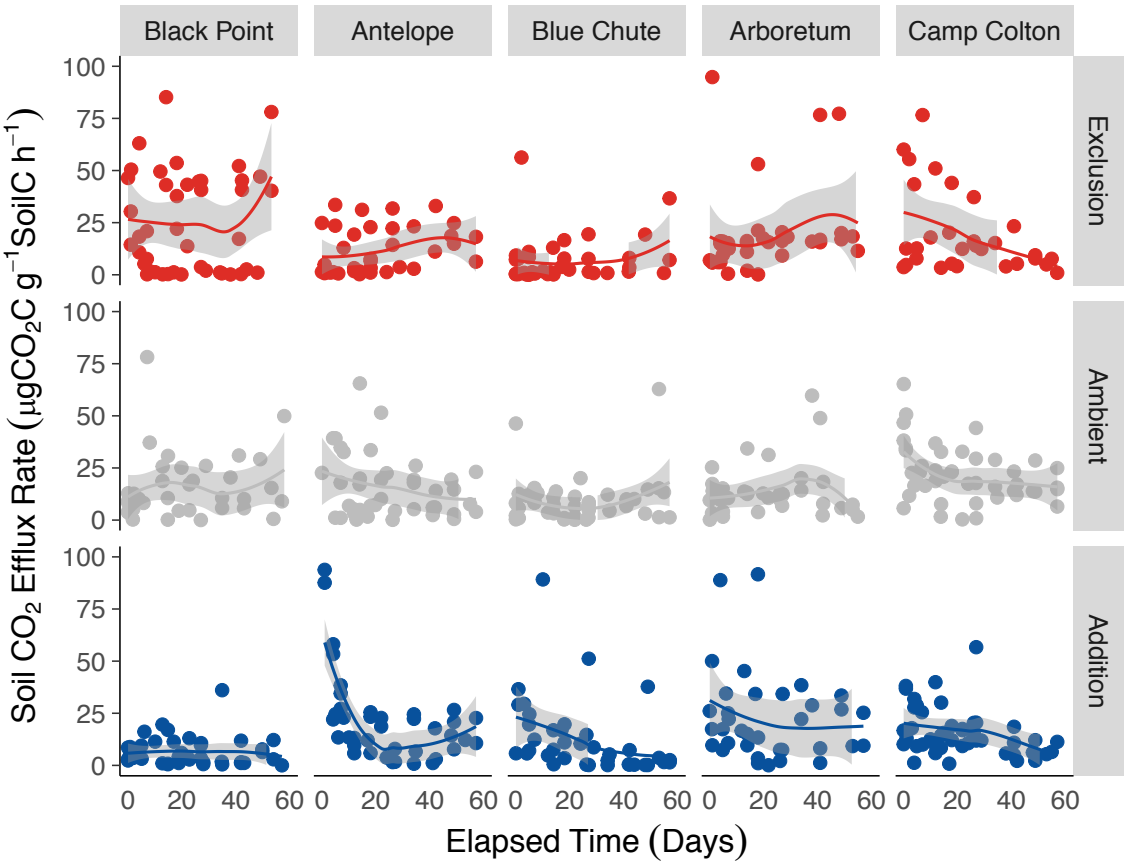

Figure 9. *Ex situ* soil CO_2 efflux rates for each plot for soil by sites increasing in elevation. Efflux rates separated by precipitation manipulation treatment, which are ambient (gray; control; no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion; red), and 50% water addition (addition; blue). Samples were taken in canopy (dominated by grass) and at a depth of 0-5 cm. Regression lines made with locally estimated scatterplot smoothing (LOESS) and a span of 1.

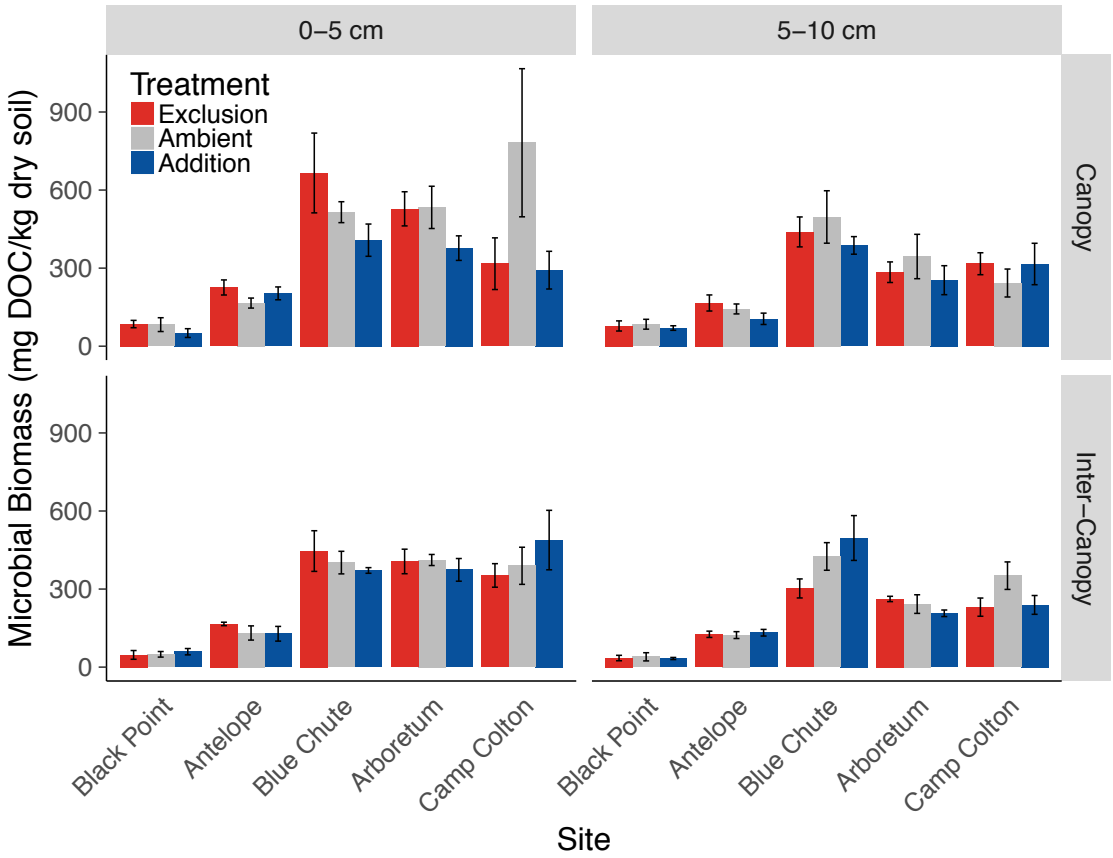

Figure 10. Microbial biomass carbon (mean ±SE) for each treatment by sites increasing in elevation. Samples were taken in canopy (dominated by grass) and inter-canopy (open spaces between dominant grasses) and at depths of 0-5 cm and 5-10 cm. The precipitation manipulation treatments are: ambient (gray; control; no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion; red), and 50% water addition (addition; blue).

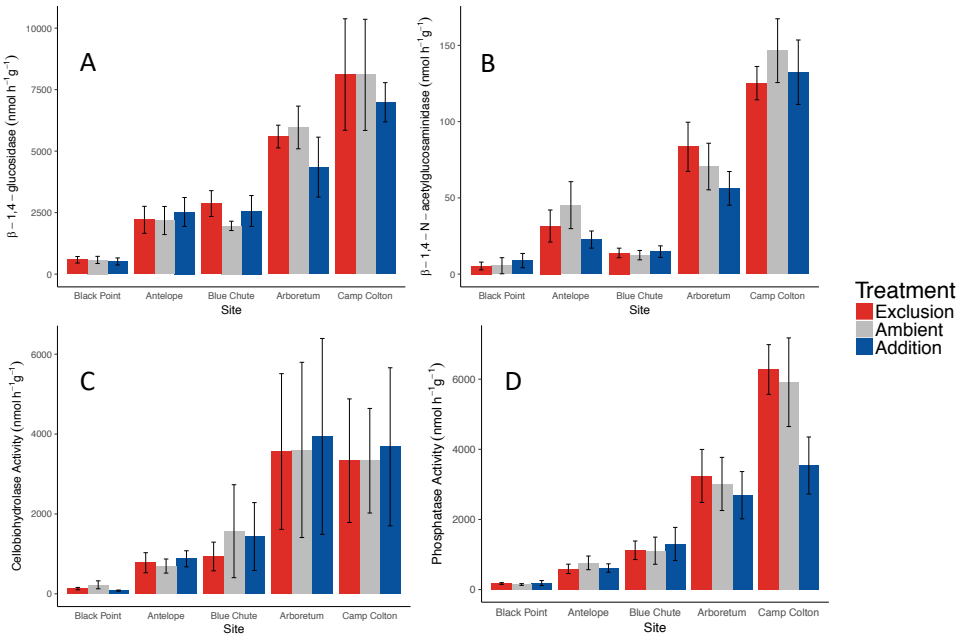

Figure 11. Activity (mean ±SE) of (A) β-1,4-glucosidase, (B) β-1,4-N-acetylglucosaminidase, (C) cellobiohydrolase, and (D) phosphatase for each treatment by sites increasing in elevation. Samples were taken in canopy (dominated by grass) and at a depth of 0-5 cm. The precipitation manipulation treatments are: ambient (gray; control; no rainfall manipulation), rainout shelters with 50% removal of precipitation (exclusion; red), and 50% water addition (addition; blue).

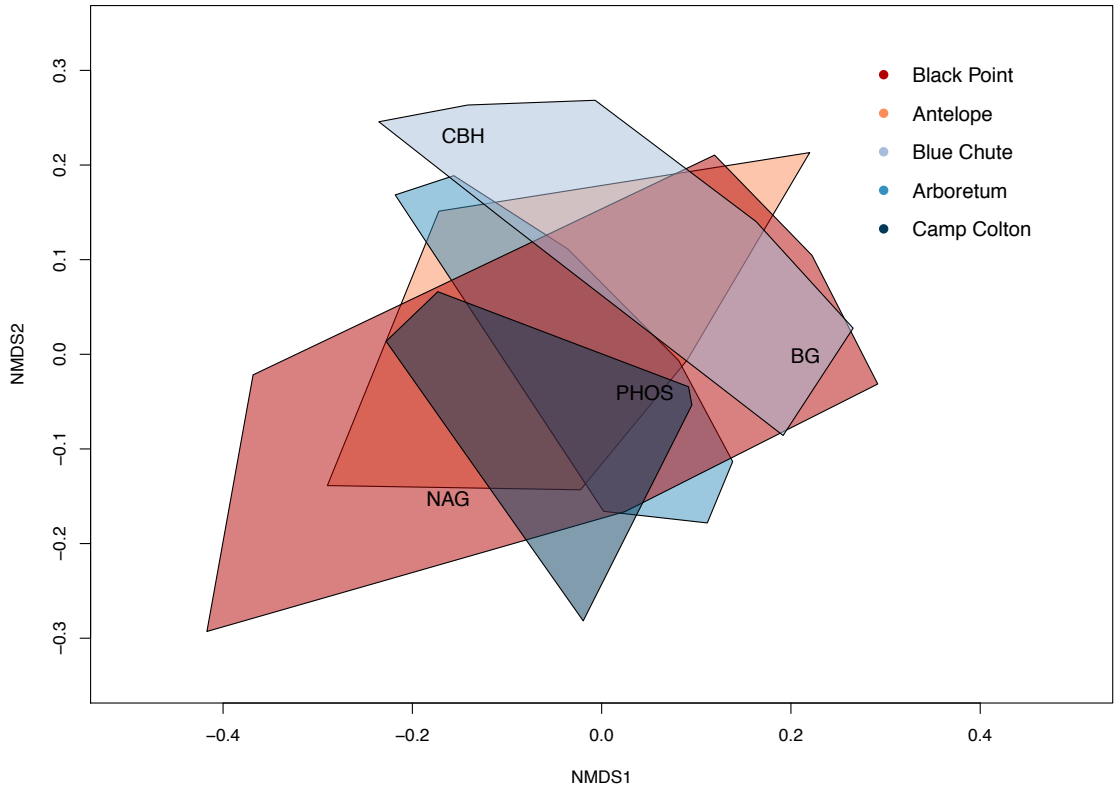

Figure 12. Nonmetric Multidimensional Scaling (NMDS) figure of sites and enzymes. The five sites are Black Point, Antelope, Blue Chute, Arboretum, and Camp Colton. The four enzymes are β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), cellobiohydrolase (CBH), and phosphatase (PHOS).

REFERENCES

Allison, S.D., Jastrow, J.D., 2006. Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. Soil Biol. Biochem. 38, 3245–3256. https://doi.org/10.1016/j.soilbio.2006.04.011

Alster, C.J., German, D.P., Lu, Y., Allison, S.D., 2013. Microbial enzymatic responses to drought and to nitrogen addition in a southern California grassland. Soil Biol. Biochem. 64, 68–79. https://doi.org/10.1016/j.soilbio.2013.03.034

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A., Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141, 221–235. https://doi.org/10.1007/s00442-004-1519-1

Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T., Zak, J.C., 2009. Linking microbial community structure and function to seasonal differences in soil moisture and temperature in a Chihuahuan Desert grassland. Microb. Ecol. 58, 827–842. https://doi.org/10.1007/s00248-009-9529-5

Chatterjee, A., Jenerette, G.D., 2011. Changes in soil respiration Q10 during drying-rewetting along a semi-arid elevation gradient. Geoderma 163, 171–177. https://doi.org/10.1016/j.geoderma.2011.04.003

Conant, R.T., Dalla-Betta, P., Klopatek, C.C., Klopatek, J.M., 2004. Controls on soil respiration in semiarid soils. Soil Biol. Biochem. 36, 945–951. https://doi.org/10.1016/j.soilbio.2004.02.013

Conant, R.T., Klopatek, J.M., Malin, R.C., Klopatek, C.C., 1998. Carbon pools and fluxes along an environmental gradient in northern Arizona. Biogeochemistry 43, 43–61. https://doi.org/10.1023/A:1006004110637

de Graaff, M.A., Throop, H.L., Verburg, P.S.J., Arnone, J.A., Campos, X., 2014. A synthesis of climate and vegetation cover effects on biogeochemical cycling in shrub-dominated drylands. Ecosystems 17, 931–945. https://doi.org/10.1007/s10021-014-9764-6

Feng, J., Wei, K., Chen, Z., Xiaotao, L., Tian, J., Wang, C., Chen, L., 2019. Coupling and decoupling of soil carbon and nutrient cycles across an aridity gradient in the drylands of northern China: evidence from ecoenzymatic stoichiometry. Global Biogeochem. Cycles 33, 559–569. https://doi.org/10.1029/2018GB006112

Gravuer, K., Eskelinen, A., 2017. Nutrient and rainfall additions shift phylogenetically estimated traits of soil microbial communities. Front. Microbiol. 8, 1–16. https://doi.org/10.3389/fmicb.2017.01271

Harris, D., Horwáth, W.R., van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J. 65, 1853–1856. https://doi.org/10.2136/sssaj2001.1853

Huang, J., Yu, H., Guan, X., Wang, G., Guo, R., 2016. Accelerated dryland expansion under climate change. Nat. Clim. Chang. 6, 166–171. https://doi.org/10.1038/nclimate2837

Jenkinson, D.S., 1966. Studies on the decomposition of plant material in soil. Evolution (N. Y). 17, 417–423. https://doi.org/10.1111/j.1365-2389.1965.tb01424.x

Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biol. Biochem. 36, 5–7. https://doi.org/10.1016/j.soilbio.2003.10.002

Jobbágy, E.G., Jackson, R.B., Biogeochemistry, S., Mar, N., 2001. The distribution of soil nutrients with depth : global patterns and the imprint of plants. Biogeochemistry 53, 51–77.

Ladwig, L.M., Sinsabaugh, R.L., Collins, S.L., Thomey, M.L., 2015. Soil enzyme responses to varying rainfall regimes in Chihuahuan Desert soils. Ecosphere 6, 1–10. https://doi.org/10.1890/ES14-00258.1

Lal, R., 2004. Carbon Sequestration in Dryland Ecosystems. Environ. Manage. 33, 528–544. https://doi.org/10.1007/s00267-003-9110-9

Lei, T., Pang, Z., Wang, X., Li, L., Fu, J., Kan, G., Zhang, X., Ding, L., Li, J., Huang, S., Shao, C., 2016. Drought and carbon cycling of grassland ecosystems under global change: a review. Water 8, no. 10:460. https://doi.org/10.3390/w8100460

Liu, W., Zhang, Z., Wan, S., 2009. Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Glob. Chang. Biol. 15, 184–195. https://doi.org/10.1111/j.1365-2486.2008.01728.x

Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., Quero, J.L., García-Gómez, M., Gallardo, A., Ulrich, W., Bowker, M.A., Arredondo, T., Barraza-Zepeda, C., Bran, D., Florentino, A., Gaitán, J., Gutiérrez, J.R., Huber-Sannwald, E., Jankju, M., Mau, R.L., Miriti, M., Naseri, K., Ospina, A., Stavi, I., Wang, D., Woods, N.N., Yuan, X., Zaady, E., Singh, B.K., 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proc. Natl. Acad. Sci. U. S. A. 112, 15684–15689. https://doi.org/10.1073/pnas.1516684112

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: Results from a meta-analysis. Ecology 93, 930–938. https://doi.org/10.1890/11-0026.1

Munson, S.M., Bunting, E.L., Bradford, J.B., Butterfield, B.J., Gremer, J.R., 2018. Plant production responses to precipitation differ along an elevation gradient and are enhanced under extremes. Ecosystems 22, 699–708. https://doi.org/10.1007/s10021-018-0296-3

Ochoa-Hueso, R., Collins, S.L., Delgado-Baquerizo, M., Hamonts, K., Pockman, W.T., Sinsabaugh, R.L., Smith, M.D., Knapp, A.K., Power, S.A., 2018. Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. Glob. Chang. Biol. 24, 2818–2827. https://doi.org/10.1111/gcb.14113

Pascale, S., Boos, W.R., Bordoni, S., Delworth, T.L., Kapnick, S.B., Murakami, H., Vecchi, G.A., Zhang, W., 2017. Weakening of the North American monsoon with global warming. Nat. Clim. Chang. 7, 806–812. https://doi.org/10.1038/nclimate3412

Paul, E.A., Harris, D., Klug, M.J., Ruess, R.W., 1999. The determination of microbial biomass, in: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 291–317.

Prăvălie, R., 2016. Drylands extent and environmental issues. A global approach. Earth-Science Rev. 161, 259–278. https://doi.org/10.1016/j.earscirev.2016.08.003

Raich, J.W., Schlesinger, W.H., 1992. The global carbon dioxide flux in soil respiration and its relationship to vegitation and climate. Tellus B Chem. Phys. Meteorol. 44, 81–99. https://doi.org/https://doi.org/10.1034/j.1600-0889.1992.t01-1-00001.x

Robertson, G Philip, Groffman, P.M., Blair, J.M., Holland, E.A., Harris, D., 1999. Soil carbon and nitrogen availability: nitrogen mineralization, nitrification, and soil respiration potentials, in: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 258–271.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function 88, 1386–1394.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7–20. https://doi.org/https://doi.org/10.1023/A:1006247623877

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Huang, H.-P., Harnik, N., Leetmaa, A., Lau, N.-C., Li, C., Velez, J., Naik, N., 2007. Model projections of an imminent transition to a more arid climate in southernwestern North America. Science (80-.). 316, 1181–1184. https://doi.org/10.1126/science.1139601

Shen, W., Reynolds, J.F., Hui, D., 2009. Responses of dryland soil respiration and soil carbon pool size to abrupt vs. gradual and individual vs. combined changes in soil temperature, precipitation, and atmospheric [CO2]: A simulation analysis. Glob. Chang. Biol. 15, 2274–2294. https://doi.org/10.1111/j.1365-2486.2009.01857.x

Shi, Z., Thomey, M.L., Mowll, W., Litvak, M., Brunsell, N.A., Collins, S.L., Pockman, W.T., Smith, M.D., Knapp, A.K., Luo, Y., 2014. Differential effects of extreme drought on production and respiration: synthesis and modeling analysis. Biogeosciences 11, 621–633. https://doi.org/10.5194/bg-11-621-2014

Sinsabaugh, R.L., Klug, M.J., Collins, H.P., Yeager, P.E., Petersen, S.O., 1999. Characterizing soil microbial communities, in: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 318–348.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Talmon, Y., Sternberg, M., Grünzweig, J.M., 2011. Impact of rainfall manipulations and biotic controls on soil respiration in Mediterranean and desert ecosystems along an aridity gradient. Glob. Chang. Biol. 17, 1108–1118. https://doi.org/10.1111/j.1365-2486.2010.02285.x

Weltzin, J.F., Loik, M.E., Schwinning, S., Williams, D.G., Fay, P.A., Haddad, B.M., Harte, J., Huxman, T.E., Knapp, A.K., Lin, G., Pockman, W.T., Shaw, M.R., Small, Er.E., Smith, M.D., Smith, S.D., Tissue, D.T., Zak, J.C., 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. Bioscience 53, 941–952. https://doi.org/10.1641/0006-3568(2003)053[0941:atrote]2.0.co;2

Wilson, J.M., Griffin, D.M., 1975. Water potential and the respiration of microorganisms in the soil. Soil Biol. Biochem. 7, 199–204. https://doi.org/10.1016/0038-0717(75)90038-3

Xu, X., Thornton, P.E., Post, W.M., 2013. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Glob. Ecol. Biogeogr. 22, 737–749. https://doi.org/10.1111/geb.12029

Yahdjian, L., Sala, O.E., 2002. A rainout shelter design for intercepting different amounts of rainfall. Oecologia 133, 95–101. https://doi.org/10.1007/s00442-002-1024-3

Zhang, N., Liu, W., Yang, H., Yu, X., Gutknecht, J.L.M., Zhang, Z., Wan, S., Ma, K., 2013. Soil microbial responses to warming and increased precipitation and their implications for ecosystem C cycling. Oecologia 173, 1125–1142. https://doi.org/10.1007/s00442-013-2685-9

Zhu, L., Xiao, Q., Cheng, H., Shi, B., Shen, Y., Li, S., 2017. Seasonal dynamics of soil microbial activity after biochar addition in a dryland maize field in North-Western China. Ecol. Eng. 104, 141–149. https://doi.org/10.1016/j.ecoleng.2017.04.026